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The genus *Neopaxillus* (*Crepidotaceae*)

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Abstract — *Neopaxillus* (*Agaricomycotina, Basidiomycota*) was formerly placed in the *Paxillaceae* (*Boletales*) by Singer. Herein we provide additional evidence that the genus is referable to the *Crepidotaceae* (*Agaricales*) and a redescription of *N. echinospermus* from recently collected material.

Key words — fungal systematics, *Melanomphalia*, neotropical fungi, *Paxillus*, *Simocybe*

Introduction

Neopaxillus Singer (1948) was erected as a monotypic genus, typified by *N. echinosporus*. The description was based on a collection by J. Rick from sandy soil in Southern Brazil that is deposited in the Farlow Herbarium, Massachusetts, USA. *Neopaxillus* was characterized as being morphologically intermediate between the *Cortinariaceae* (*Agaricales*) and the *Paxillaceae* (*Boletales*). The habit and trama were likened to that of the *Paxillaceae* in having a clitocyboid-omphalinoid facies reminiscent of a small *Phylloporus rhodoxanthus* (Schwein.) Bres. (*Boletaceae*) or *Paxillus* species. The basidiospores, however, were described as having characters more reminiscent of *Cortinarius,* although their ornamentation was not identical to that of any known *Cortinarius* species (Singer 1948).

Soon after his 1948 publication, Singer (1951) determined that *Neopaxillus echinosporus* was in fact conspecific with the Argentine *Naucoria echinosperma* (Spegazzini 1889), which he recombined as *Neopaxillus echinospermus.* Because this name has priority, *Neopaxillus echinospermus* is the correct name for the combined taxon; however, the genus *Neopaxillus* remains typified by *N. echinosporus*. In later works, Singer included two other South American species, *Flammula echinospora* (Spegazzini 1902) and *Flammula papillosispora* (Spegazzini 1919), in the synonymy of *Neopaxillus echinospermus* (Singer 1952, 1964; Singer et al. 1990).

Singer (1948) likened *Neopaxillus echinosporus* in size to *Deconica crobula* (Fr.) Romagn., with which Rick apparently confused his collection. One distinguishing feature was the ornamented spores, which Singer regarded as a link to *Ripartites* P. Karst., a genus also then placed in the *Paxillaceae*. Their ornamented basidiospores separated both genera from all the other taxa that Singer accepted in the *Paxillaceae* (Singer 1990). *Neopaxillus* is separable from *Ripartites* by its larger basidiospores, lamellae that anastomose towards the stipe, and an almost continuous trichodermial pileipellis composed of chains of hyphae with broadly clavate terminal cells. Furthermore, according to Singer (1948), the hymenophoral trama in *Neopaxillus* is not characteristically divergent but rather weakly or vaguely bilateral in immature specimens.

It has more recently become apparent that *Paxillaceae* sensu Singer (1986) is an artificial family with many disparate constituent elements, e.g. *Omphalotus* Fayod (now placed in the *Marasmiaceae;* Kirk et al. 2008), *Hygrophoropsis* (J. Schröt.) Maire ex Martin-Sans (now placed in the *Hygrophoropsidaceae;* Kirk et al. 2008), and *Phyllobolites* Singer (now placed in the *Boletaceae;* Kirk et al. 2008). The genus *Paxillus* sensu Singer itself has been subdivided with saprobic species [e.g., *Paxillus panuoides* (Fr.) Fr.] now placed in *Tapinella* E.-J. Gilbert (*Tapinellaceae*), southern hemisphere species now in *Austropaxillus* Bresinsky & Jarosch (*Serpulaceae*), and the ectomycorrhizal members remaining in *Paxillus* (Kirk et al. 2008, Watling 2008).

Ripartites, which has been placed in several agaric families, including the *Tricholomataceae* where it is presently placed, was linked to *Neopaxillus* by general appearance, with the pair placed in proximity to *Paxillus* by virtue of the open-pored hilum of the basidiospore. Results from numerical analyses conducted by Machol & Singer (1971) supported this placement. However, in his review of the boletes and their relatives, Watling (2008) deemed it necessary to reexamine *Neopaxillus,* which became possible with collection of new material in the Atlantic rainforests of Brazil by André de Meijer. Watling's initial examination of this material indicated a possible link to the *Crepidotaceae* due to the echinulate basidiospores reminiscent of similar ornamentation seen in some *Crepidotus* taxa. This observation led us to attempt to infer the true position of *Neopaxillus* within the *Agaricomycotina* using DNA sequence analyses.

Materials & methods

Collections examined

New collections of *N. echinospermus* were made by André de Meijer on forest humus in the Mata Atlantica in Paraná, Brazil. Material is deposited in the Royal Botanic Garden, Edinburgh, Scotland, UK (E). The field colors were matched to Methuen's Handbook (Kornerup & Wanscher 1978). Microscopic characters were observed using a Leitz Ortholux microscope and examined in a 10% aqueous ammonia solution.

25 spores of each collection in five separate mounts were measured; Melzer's reagent (a mixture of chloral hydrate, potassium iodide and elemental iodine) and cotton blue in lactophenol supplemented the observations; material examined using a Jeol T200 scanning electron microscope were suspended first in the above ammonia solution before applying to the stub, drying, and coating with gold.

Molecular analyses

DNA was extracted from dried tissue from the two herbarium *Neopaxillus echinospermus* specimens with the UltraClean Plant DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA) following the manufacturer's instructions. Primers LSU4-B (Aime & Phillips-Mora 2005) and LR6 (Moncalvo et al. 1995) were used to amplify and sequence the first ~1200 bp of the 28S ribosomal DNA using previously described parameters (Aime & Phillips-Mora 2005). Preliminary phylogenetic analysis was conducted by analyzing LSU sequences of *N. echinospermus* within the 877 taxa dataset of Moncalvo et al. (2002). This dataset contains representatives of all major agaricalean lineages, as well as exemplar taxa from the other major *Agaricomycotina* clades, including *Boletales*, with *Auricularia polytricha* (*Auriculariales*) as the outgroup (Moncalvo et al. 2002). Based on the results of the preliminary analyses (not shown) a new dataset was constructed with additional sampling from within the *Crepidotaceae*, to which *N. echinospermus* showed affinity. A total of 21 species were chosen including the type species of *Crepidotus* [*C. mollis* (Schaeff.) Staude] and *Simocybe* [type, *S. centunculus* (Fr.) P. Karst.] from previously published *Crepidotaceae* sequences (Aime & Miller 2002, Aime et al. 2002, Aime et al. 2005, Aime et al. 2009, Matheny 2005, Moncalvo et al. 2002, and unpublished), exemplar sequences from the genus *Inocybe* (Matheny 2005, Moncalvo et al. 2002, Ryberg et al. 2008, and unpublished), which has been suggested as the sister family to the *Crepidotaceae* sensu Aime (Matheny et al. 2006), with *Agaricus* aff. *campestris* and *Deconica montana* (=*Psilocybe montana*) (Matheny et al. 2006, and unpublished) selected as outgroups.

New sequences were edited and assembled in Sequencher v.4.1.4 (Gene Codes Corp., Ann Arbor, MI) and aligned visually in Se-Al v2.0a11 (Andrew Rambaut, Dept. Zoology, University of Oxford, U.K.; http://evolve.zoo.ox.ac.uk/) over a total of 1074 bp. Maximum likelihood (ML) analyses were conducted in RAxML-HPC2 v. 7.2.7 (Stamatakis 2006) via the CIPRES portal (Miller et al. 2011) using default parameters and 1000 bootstrapping replicates. Maximum parsimony (MP) analyses were conducted in Paup* v4.0b10 (Swofford 2002) as heuristic searches with 1000 random addition replicates and TBR branch swapping; bootstrap analysis consisted of 1000 MP replicates. Sequences have been deposited in GenBank as JQ404442 (AM 1424) and JQ404443 (AM 1690).

Results

Based on prior molecular studies, the members of *Paxillaceae* sensu Singer have been segregated into three groups, the *Paxillineae*, *Serpulaceae* (*Coniophorineae*), and *Tapinellineae*, all of which belong to the *Boletales* (Binder & Hibbett 2006). Preliminary results from 28S analyses of *Neopaxillus echinospermus* within an inclusive dataset of *Agaricomycotina* placed

N. echinospermus firmly within the *Crepidotaceae*, with no affinity to the *Boletales* (data not shown). A second dataset was constructed with exemplars from all *Crepidotaceae* genera and representative outgroups (Fig. 1), strongly supporting the placement of *N. echinospermus* within the *Crepidotaceae*. The 28S locus, alone, did not provide strong support separating the three genera (*Crepidotus*, *Simocybe*, and *Neopaxillus*), although each forms a monophyletic clade within *Crepidotaceae* under all searching algorithms used, with *Neopaxillus* consistently resolved as the sister lineage to *Crepidotus*.

Taxonomy

Neopaxillus echinospermus (Speg.) Singer, Lilloa 22: 633. 1951 ["1949"].

≡ *Naucoria echinosperma* Speg., Bol. Acad. Nac. Cienc. Cordoba 11: 424. 1889.

= *Flammula echinospora* Speg., An. Mus. Nac. B.Aires 8: 51. 1902.

= *Flammula papillosispora* Speg., Bol. Acad. Nac. Cienc. Cordoba 23:396. 1919.

= *Neopaxillus echinosporus* Singer, Mycologia 40: 262. 1948.

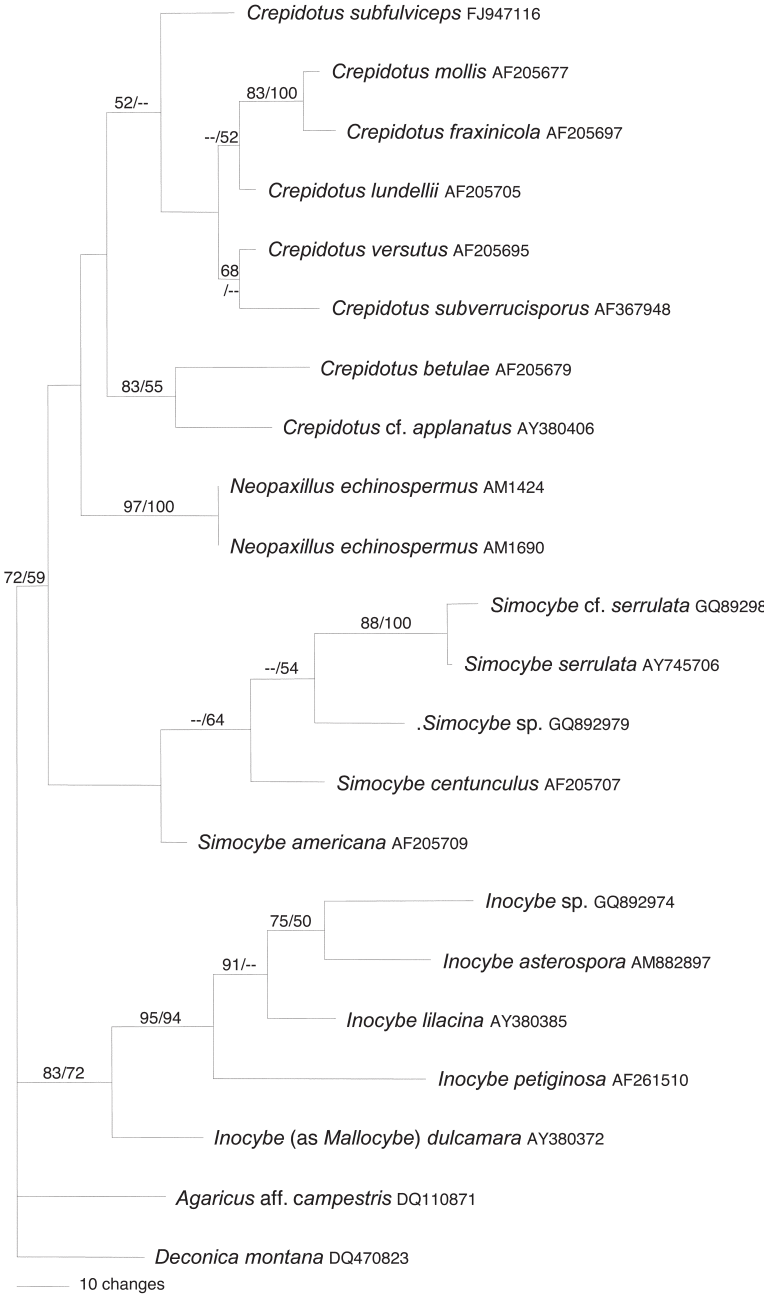
Pileus 12–40 mm broad, center depressed even in youth, slightly hygrophanous, non-striate when fresh, golden brown, brownish orange when dry, smooth, dry, glabrous; margin often decurved, sometimes undulate. Lamellae strongly decurrent, distant, margin straight or concave, 5.5 mm broad, clay-brown at first, dark brown when fully mature, neither interveined, nor forked. Stipe 17–40 × 3.5–7 mm, cylindrical or slightly attenuated downwards, solid, paler and more yellow than pileus, minutely innately brown-striate, smooth, dry; basal mycelium white. Context in pileus when fresh yellowish brown, drying pale ochraceous to white; in stipe drying white, but in stipe base often becoming brown; odor fungoid or none.

Basidiospores 7–8(–8.5) µm subglobose, strongly warty-echinulate, ornaments <0.7 µm high, inamyloid. Cheilo- & pleurocystidia absent. Pileipellis composed of irregularly arranged, rarely encrusted hyphae 5–7 µm broad. Clamp-connections absent.

Material examined: **BRAZIL**, Paraná, General Caneiro, in forest humus, 20 xii 1989, Meijer 1424 (E); 12 v 1990, Meijer 1690 (E).

Notes: There is some confusion as to where João Rick collected his material, which Watling & de Meijer (1997) have addressed. This fungus has been previously illustrated (e.g., de Meijer 2008, fruiting body; Watling & de Meijer 1997, basidiospores) and described in detail (e.g., Singer 1948, 1952, Watling & de Meijer 1997).

Fig. 1. Phylogenetic tree based on maximum parsimony (MP) analyses of 28S ribosomal DNA sequences of two isolates of *Neopaxillus echinospermus* with representative crepidotaceous and inocybaceous taxa. *Agaricus* aff. *campestris* and *Deconica montana* chosen as outgroups. Support at nodes shown as MP bootstrapping/maximum likelihood bootstrapping values.


Crepidotus subfulviceps FJ947116
Crepidotus mollis AF205677
83/100
52/--
Crepidotus fraxinicola AF205697
--/52
Crepidotus lundellii AF205705
Crepidotus versutus AF205695
68
/--
Crepidotus subverrucisporus AF367948
Crepidotus betulae AF205679
83/55
Crepidotus cf. *applanatus* AY380406
Neopaxillus echinospermus AM1424
97/100
Neopaxillus echinospermus AM1690
72/59
Simocybe cf. *serrulata* GQ89298
88/100
Simocybe serrulata AY745706
--/54
.*Simocybe* sp. GQ892979
--/64
Simocybe centunculus AF205707
Simocybe americana AF205709
Inocybe sp. GQ892974
75/50
Inocybe asterospora AM882897
91/--
Inocybe lilacina AY380385
95/94
83/72
Inocybe petiginosa AF261510
Inocybe (as *Mallocybe*) *dulcamara* AY380372
Agaricus aff. *campestris* DQ110871
Deconica montana DQ470823
10 changes

Discussion

Previous work showed the *Crepidotaceae* to consist of two saprobic agaricoid genera — the predominantly pleurotoid genus *Crepidotus* (Fr.) Staude and *Simocybe* P. Karst. (which contains both pleurotoid and centrally stipitate forms; Aime et al. 2005) — and several cyphelloid genera (Singer 1986, Bodensteiner et al. 2004). Our results support the findings of Vizzini et al. (2012) in placing *Neopaxillus* as a sister genus to *Crepidotus.* However, that study was inconclusive in its treatment of *Simocybe*, whereas the latter is supported within *Crepidotaceae* in our analyses. The inclusion of *Neopaxillus* expands the familial concept to include a genus composed entirely of centrally stipitate, terrestrial species with unknown ecology. Although some species of *Crepidotus* and *Simocybe* can be found on bare or moss-covered soil, they generally fruit on well decomposed large diameter logs, as well as fallen branches and sometimes herbaceous and twiggy material. One question that has not been resolved is whether *Neopaxillus* is ectomycorrhizal, although its present affinities would not suggest this life-strategy, and at least one other species (i.e., *N. plumbeus* Singer & Lodge) was not collected near any ectomycorrhizal host trees (D. Jean Lodge, pers. comm.). However, the sister-relationship between *Crepidotaceae* and the ectomycorrhizal *Inocybaceae* (Matheny et al. 2006) admits the possibility that *Neopaxillus* is also ectomycorrhizal. A placement in *Paxillaceae* might have hinted at an ectomycorrhizal role, although in the sense of Singer (1951) this family contained both saprobes and ectomycorrhizal associates. Singer (1952) wrote "on the ground in woods accompanying rivers usually in small groups but never densely clustered." Our present collections are from the Atlantic rainforest in mixed ombrophilous forest with *Araucaria angustifolia* at 800–900 m above sea level, which basically agrees with Singer's observation for Argentina. He also noted, "fruiting in summer and fall." Watling & de Meijer (1997) indicated that de Meijer's collections occurred between December (summer) and May (fall) in Brazil.

A hint to the relationship of *Neopaxillus* and the *Crepidotaceae* may be sought in the observations of Singer, who noted "an actual affinity between *Neopaxillus* and *Tubaria* section *Thermophila*, but even so, they are not congeneric, but can be put into the same family" (Singer 1951). Indeed, *Tubaria thermophila* has subsequently been shown to belong to *Crepidotaceae*, along with several other stipitate species formerly placed in *Melanomphalia* M.P. Christ. (Aime et al. 2002, 2005, 2009). This refuted Christiansen's suggestion that *Melanomphalia* might be related to the *Gomphidiaceae* (Christiansen 1936), which, like *Paxillaceae*, is now also placed within *Boletales*. Horak (1980) also hinted at a relationship between *Neopaxillus* and *Crepidotus* in his descriptions of *N. bryogenus* E. Horak and *C. velutinoaffinis* Singer, both agarics from South America, although spore ornamentation is very different between the former

and the majority of *Crepidotus* spp. Indeed the illustrations in Horak (1980) for both species resemble those depicted for *Melanomphalia inocyboides* Singer and *M. viscosa* Singer, respectively, which leaves one to suspect that *N. bryogenus* may not be assignable to *Neopaxillus* based on the type species. Three additional *Neopaxillus* species are known: *N. plumbeus* from Puerto Rico (Singer & Lodge 1989), *N. reticulatus* (Petch) Pegler from Sri Lanka (Pegler 1986), and *N. dominicanus* Angilini & Vizzini from the Dominican Republic and Mexico.

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